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TOPICAL HAZARD EVALUATION PROGRAM PROCEDURAL GUIDE.(U)
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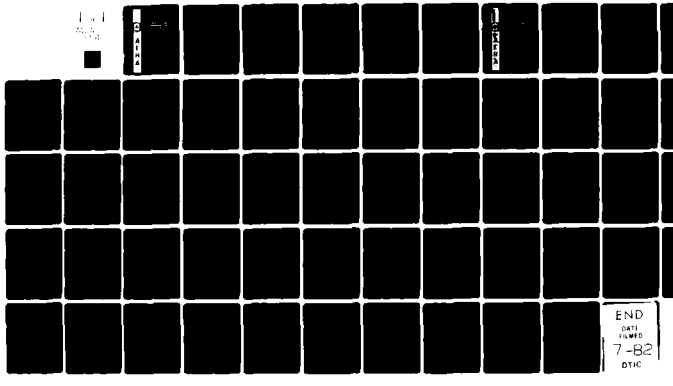

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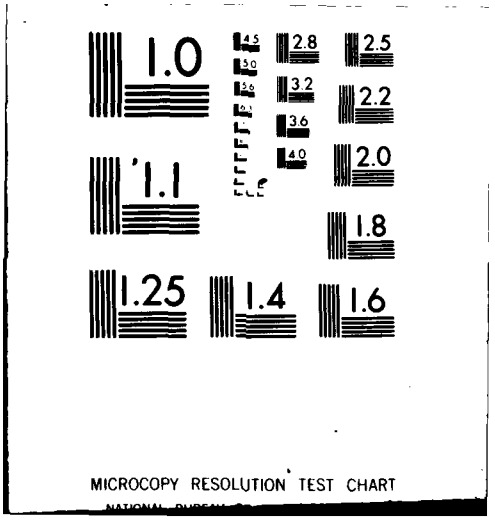
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UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010

TOXICOLOGY DIVISION
TOPICAL HAZARD EVALUATION PROGRAM
PROCEDURAL GUIDE

JANUARY 1982

TOPICAL HAZARD EVALUATION PROGRAM

PROCEDURAL GUIDE

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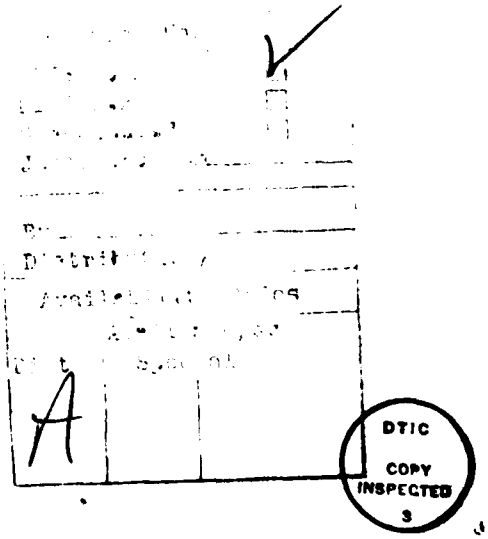


DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

REPLY TO
ATTENTION OF

DEPARTMENT OF THE ARMY
US ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT

December 1981

STANDING OPERATING PROCEDURE

TOPICAL HAZARD EVALUATION PROGRAM

1. PURPOSE. To provide guidance for further entomological testing of candidate insect repellents.

2. SCOPE. This standing operating procedure (SOP) is compiled for use in the animal facilities of the Toxicology Division, US Army Environmental Hygiene Agency (USAEHA) and is to be endorsed and periodically revised by the Animal Use Review Committee, USAEHA, and the Chief, Analytical Quality Assurance Office, USAEHA, and approved by the Chief, Toxicology Division.

3. REFERENCES.

a. Title 21, Code of Federal Regulations, (CFR) 1981 rev., Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

b. Memorandum of Understanding between USAEHA; USA Health Services Command; DA, Office of the Surgeon General; Armed Forces Pest Control Board; Department of Agriculture, Agricultural Research, Science and Education Administrations, titled, Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

c. SOP, HSE-LT/WP, this Agency, subject: Animal Facilities, Toxicology Division Buildings E2100 and E2101.

d. SOP, HSE-LT/WP, this Agency, subject: Individual Animal Identification.

e. SOP, HSE-LT/WP, this Agency, subject: Primary Dermal Irritation Study.

f. SOP, HSE-LT/WP, this Agency, subject: Primary Eye Irritation Study.

g. SOP, HSE-LT/WP, this Agency, subject: Primary Dermal Photochemical Skin Irritation Study.

h. SOP, HSE-LT/WP, this Agency, subject: Oral Approximate Lethal Dose (ALD) Procedure.

i. SOP, HSE-LT/WP, this Agency, subject: Guinea Pig Sensitization Test.

4. SAMPLE HANDLING PROCEDURES.

a. Samples are usually received via the mail from the Department of Agriculture. Upon receipt the samples are assigned project numbers and file folders are assembled by the division secretary.

b. A letter is written to the sender acknowledging sample receipt.

c. The samples are then given to the designated division sample control officer. This individual will log the samples into his notebook, and record the volume and/or weight of the sample received and date of receipt.

d. The project number and unique USDA sample number are recorded in the Topical Hazard Evaluation Program (THEP) Laboratory Notebook No. 10.

e. A disposition form (DF) is written requesting an infrared scan from the Organic Environmental Chemistry Division (OECD), USAEHA. The samples and the DF are sent together to OECD thru the Analytical Quality Assurance Office, USAEHA.

f. When the samples are returned from OECD, they are stored in room 3202 until needed.

5. TESTING PROCEDURES.

a. The animals for testing are assigned unique numbers.

b. The animals for test are recorded along with project and sample number in Laboratory Notebook No. 13 for eye, skin, and photochemical irritations, No. 48 for guinea pig sensitization and No. 72 for ALD.

c. Testing order is at the discretion of the investigator but it is usually done in the following order: ALD, primary dermal irritation, primary eye irritation, photochemical skin irritation and guinea pig sensitization (GPST).

d. Raw data is recorded on the appropriate forms and filed in project folder after investigator signs and dates it.

e. The final USAEHA toxicity category is also recorded in Laboratory Notebook No. 10.

6. REPORTING PROCEDURES.

a. All samples are accepted for further testing as candidate insect repellents except if they are in the following USAEHA toxicity categories any one of which is cause for rejection.

ALD	500 mg/kg or less
SKIN	Category III, IV or V
EYE	Category E or F
PHOTO	Photochemical irritant
GPST	20% of animals sensitized

b. An Agency report is written for all samples whether accepted or rejected in the style as shown in the sample report (Appendix A).

c. Copies of the final report are mailed according to the listed distribution on the report's cover letter.

d. Extra copies are maintained in the Toxicology Division Office.

7. APPROVALS.

a. This SOP is in accordance with 21 CFR 58 and has been reviewed and approved by the USAEHA Animal Use Review Committee

Mack A. Holt

MACK A. HOLT, DVM
CPT(P), VC
Chairman, Animal Use Review Committee

b. This SOP has been reviewed and approved by the USAEHA Analytical Quality Assurance Office. The Analytical Quality Assurance Office inspects each phase of an in-process study of this type to assure that no significant problems exist that are likely to affect the integrity of the study.

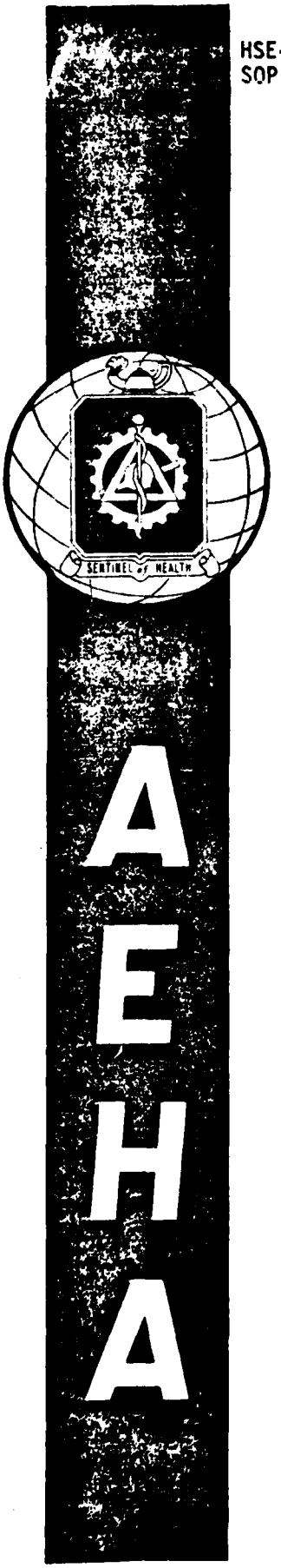
PAUL V. SNEERINGER, Ph.D.
Chief, Analytical Quality
Assurance Office

c. Designated Toxicology Division personnel will be responsible for the performance of this Topical Hazard Evaluation Program SOP.

ARTHUR H. McCREESH, Ph.D.
Chief, Toxicology Division

d. This Topical Hazard Evaluation Program SOP was prepared by:

MICHAEL J. TOPPER, DVM
CPT, VC
Laboratory Animal Veterinary Officer
Toxicology Division



HSE-LT
SOP, Topical Hazard Evaluation Program

APPENDIX A

UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010

TOPICAL HAZARD EVALUATION PROGRAM OF CANDIDATE INSECT REPELLENTS
US DEPARTMENT OF AGRICULTURE PROPRIETARY CHEMICALS
STUDY NUMBERS 75-51-0182-82 thru 75-51-0189-82, 75-51-0192-82,
and 75-51-0242-82
OCTOBER 1978 - SEPTEMBER 1981

Approved for public release, distribution unlimited.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 75-51-0182-82 thru 75-51-0189-82, 75-51-0192-82 and 75-51-0242-82 2. GOVT ACCESSION NO. AD-A115 418	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) THEP of Candidate Insect Repellents, US Department of Agriculture Proprietary Chemicals Study Nos. 75-51-0182-82 thru 75-51-0189-82, 75-51-0192-82, and 75-51-0242-82, Oct 78 - Sep 82	5. TYPE OF REPORT & PERIOD COVERED Final, Oct 78 - Sep 81 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Michael J. Topper, CPT, VC John G. Harvey, Jr.	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Environmental Hygiene Agency Aberdeen Proving Ground, MD 21010	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Commander US Army Health Services Command Fort San Houston, TX 78234	12. REPORT DATE Oct 78 - Sep 81 13. NUMBER OF PAGES 8
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

AI3-37565	AI3-37571	Skin Irritation	USDA Proprietary Chemicals
AI3-37566	AI3-37572	Eye Irritation	Topical Hazard Evaluation
AI3-37567	AI3-37574	ALD	Program
AI3-37569	AI3-37578	Photo Irritation	
AI3-37570	AI3-38010	Guinea Pig Sensitization	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Preliminary hazard evaluations of the above candidate insect repellent chemicals were performed by means of laboratory animal studies using rats, rabbits, and guinea pigs. Chemicals AI3-37565,37567,37569,37570,37571,37572,37574 and 38010 did not cause any skin irritation. Chemical AI3-37566 and 37578 caused mild primary skin irritation. Chemical AI3-37574 was noninjurious to the eyes of rabbits. Chemicals AI3-37565 and 37572 caused mild injury to the cornea and chemicals AI3-37566, 37567,37569,37570,37571,37578, and 38010 caused mild injury to the cornea and, in addition, some injury to the conjunctiva.

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

All chemicals were relatively nontoxic by ingestion and did not cause photo-irritation or prove to be skin sensitizers. Chemicals AI3-37570 and 37574 demonstrated some skin irritation from ethanol solutions during photoirritation studies. It was recommended that all chemicals be approved for further testing as candidate insect repellents.

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

CPT Topper/ldr/AUTOVON
584-3980

9 DEC 1981

REPLY TO ATTENTION OF
HSE-LT-T/WP

SUBJECT: Topical Hazard Evaluation Program of Candidate Insect Repellents, US Department of Agriculture Proprietary Chemicals, Study Numbers 75-51-0182-82 thru 75-51-0189-82, 75-51-0192-82, and 75-51-0242-82, October 1978 - September 1981

Executive Secretary
Armed Forces Pest Management Board
Forest Glen Section, WRAMC
Washington, DC 20012

A summary of the pertinent findings and recommendations of the inclosed report follows:

Preliminary hazard evaluations of the above candidate insect repellent chemicals were performed by means of laboratory animal studies using rats, rabbits, and guinea pigs. Chemicals AI3-37565, 37567, 37569, 37570, 37571, 37572, 37574, and 38010 did not cause any skin irritation. Chemicals AI3-37566 and 37578 caused mild primary skin irritation. Chemical AI3-37574 was noninjurious to the eyes of rabbits. Chemicals AI3-37565 and 37572 caused mild injury to the cornea, and chemicals AI3-37566, 37567, 37569, 37570, 37571, 37578, and 38010 caused mild injury to the cornea and, in addition, some injury to the conjunctiva. All chemicals were relatively nontoxic by ingestion and did not cause photoirritation or prove to be skin sensitizers. Chemicals AI3-37570 and 37574 demonstrated some skin irritation from ethanol solutions during photoirritation studies. It was recommended that all chemicals be approved for further testing as candidate insect repellents.

FOR THE COMMANDER:

JOHN F. MAZUR
LTC, MSC
Director, Laboratory Services

1 Incl
as (5 cy)

CF:
HQDA (DASG-PSP)
Cdr, HSC (HSPA-P)
Dir, Advisory Cen on Tox, NRC
Comdt, AHS (HSA-IPM)
USDA, ARS (Dr. Terrence McGovern)
USDA, ARS-Southern Region (2 cy)

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

REPLY TO ATTENTION OF
HSE-LT-T/WP

TOPICAL HAZARD EVALUATION PROGRAM OF CANDIDATE INSECT REPELLENTS
US DEPARTMENT OF AGRICULTURE PROPRIETARY CHEMICALS
STUDY NUMBERS 75-51-0182-82 thru 75-51-0189-82, 75-51-0192-82,
and 75-51-0242-82
OCTOBER 1978 - SEPTEMBER 1981

1. AUTHORITY.

a. Letter, US Department of Agriculture - Agricultural Research Service, Southern Region, Insects Affecting Man and Animal Research Laboratory, Gainesville, FL, 13 October 1978 (AI3-37565, 37566, 37567, 37569, 37570, 37571, 37572, 37574, and 37578).

b. Letter, US Department of Agriculture - Agricultural Research Service, Southern Region, Insects Affecting Man and Animal Research Laboratory, Gainesville, FL, 23 November 1978 (AI3-38010).

c. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the Department of Agriculture, Agricultural Research, Science and Education Administrations, titled, Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

2. REFERENCE. Toxicology Division Standing Operating Procedures, US Army Environmental Hygiene Agency (USAEHA), 1981.

3. PURPOSE. The purpose of this program is to provide guidance for further entomological testing of the candidate insect repellents: AI3-37565, 37566, 37567, 37569, 37570, 37571, 37572, 37574, 37578, and 38010, US Department of Agriculture (USDA) Proprietary Chemicals.

4. SUMMARY OF FINDINGS. Hazard evaluations of the above-named candidate repellents were conducted by this Agency using New Zealand White rabbits for skin and eye studies, Hartley guinea pigs for a skin sensitization study, and Sprague-Dawley rats for determination of oral toxicity. A tabular presentation of animal toxicity data developed in this Agency follows:*†

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* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education and Welfare Publication No. (NIH) 74-23, revised 1978.
† The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

TABLE. PRESENTATION OF DATA

Test	Results	Interpretation
SKIN IRRITATION STUDIES		
Rabbits		
Single 24-hour application to intact and abraded skin of New Zealand White rabbits. 0.5 mL technical grade chemical applied to each of six rabbits.	Chemicals AI3-37565, 37567, 37569, 37570, 37571, 37572, 37574, and 38010 did not cause any irritation of the intact skin or of the skin surrounding an abrasion.	USAEHA Category I (ref Appendix A)
	Chemicals AI3-37566 and 37578 produced mild primary irritation of the intact skin and the skin surrounding an abrasion.	USAEHA Category II (ref Appendix A)
EYE IRRITATION STUDIES		
Rabbits		
Single 24-hour application of 0.1 mL technical grade chemical to one eye of each of six New Zealand White rabbits.	Chemical AI3-37574 did not cause any irritation to the eyes of rabbits.	USAEHA Category A (ref Appendix A)
	Chemicals AI3-37565 and 37572 caused mild injury to the cornea.	USAEHA Category B (ref Appendix A)
	Chemicals AI3-37566, 37567, 37569, 37570, 37571, 37578, and 38010 caused mild injury to the cornea and, in addition, some injury to the conjunctiva.	USAEHA Category C (ref Appendix A)

Test	Results	Interpretation
APPROXIMATE LETHAL DOSE (ALD)		
Oral		
Rats (male)-no diluent	AI3-37565 4300 mg/kg AI3-37566 9700 mg/kg AI3-37567 9700 mg/kg AI3-37569 9700 mg/kg AI3-37570 6500 mg/kg AI3-37571 6500 mg/kg AI3-37572 2900 mg/kg AI3-37574 2900 mg/kg AI3-37578 2900 mg/kg AI3-38010 6400 mg/kg	These chemicals are relatively nontoxic by ingestion.
PHOTOCHEMICAL SKIN IRRITATION STUDIES		
Rabbits		
A single 0.05 mL application of a 25-percent (w/v) solution of each chemical and a 10-percent (w/v) Oil of Bergamot solution (positive control) in 95 percent ethyl alcohol were applied to the intact skin of six rabbits. Five minutes after application, the rabbits were exposed to UV light (365 nm) for 30 minutes at a distance of 10-15 cm.	A 25-percent solution of each tested chemical in ethanol did not cause a photochemical irritation reaction under test conditions.	All tested chemicals did not cause a photochemical irritation reaction under test conditions and are not expected to cause a photochemical irritation in humans.
	Ethanol solutions of AI3-37570 and 37574 caused slight irritation at both UV and non-UV skin sites.	Ethanol solutions of AI3-37570 and 37574 may cause skin irritation in some sensitive individuals. Personnel experiencing this reaction should wash off the solution as soon as possible.

Test	Results	Interpretation
Control		
Following UV exposures of the rabbits, 0.05 mL of test chemical, positive control, and diluent were applied to additional skin areas to serve as unirradiated control sites. Application areas were checked for skin irritation at 24, 48, and 72 hours.	Positive control application and irradiation caused greater irritant effects than in unirradiated skin areas.	
SENSITIZATION STUDIES		
Guinea Pigs (Male)		
Intradermal injections of 0.1 mL of a 0.1-percent solution (w/v) of the tested chemicals or of dinitrochlorobenzene (DNCB)* in a mixture containing 1 volume of propylene glycol and 29 volumes of saline.		
Ten test guinea pigs for each chemical were given ten sensitizing doses over a 3-week period. After 2 weeks rest, they were challenged with intradermal (ID) injections of each test chemical.	Challenge doses of the tested chemicals did not produce a sensitization reaction.	The tested chemicals did not produce sensitization reactions under test conditions and are not expected to produce sensitization reactions in man.
Ten positive control guinea pigs were sensitized over 3 weeks with DNCB. After 2 weeks rest, they were challenged with ID injections of DNCB.	Challenge dose of DNCB in positive control guinea pigs produced a marked sensitization reaction in 10 out of 10 guinea pigs.	DNCB produced a marked reaction, indicating the guinea pigs responded to sensitizing agents.

* A known skin sensitizer.

5. CONCLUSION. Chemicals AI3-37565, 37567, 37569, 37570, 37571, 37572, 37574, and 38010 did not cause any skin irritation. Chemicals AI3-37566 and 37578 caused mild primary skin irritation. Chemical AI3-37574 was noninjurious to the eyes of rabbits. Chemicals AI3-37565 and 37572 caused mild injury to the cornea, and chemicals AI3-37566, 37567, 37569, 37570, 37571, 37578, and 38010 caused mild injury to the cornea and, in addition, some injury to the conjunctiva. All chemicals were relatively nontoxic by ingestion and did not cause photoirritation or prove to be skin sensitizers. Chemicals AI3-37570 and 37574 demonstrated some skin irritation from ethanol solutions during photoirritation studies.

6. RECOMMENDATION. Under the provisions of the Memorandum of Understanding (paragraph 1c), it is recommended that the following USDA proprietary chemicals be approved for further testing as candidate insect repellents: AI3-37565, 37566, 37567, 37569, 37570, 37571, 37572, 37574, 37578, and 38010. Ethanol solutions of chemicals AI3-37570 and 37574 may cause skin irritation in sensitive individuals and, if experienced, the site should be washed with copious amounts of water.

Michael J. Topper

MICHAEL J. TOPPER, DVM
CPT, VC
Laboratory Animal Veterinary Officer
Toxicology Division

John G. Harvey Jr

JOHN G. HARVEY, JR
Biological Laboratory Technician
Toxicology Division

APPROVED:

for Mack A. Holt

ARTHUR H. McCREESH, Ph.D.
Chief, Toxicology Division

APPENDIX A

TOPICAL HAZARD EVALUATION PROGRAM
DEFINITIONS OF CATEGORIES OF COMPOUNDS BEING CONSIDERED FOR ACUTE SKIN APPLICATION

CATEGORY I - Compounds producing no primary irritation of the intact skin or no greater than mild primary irritation of the skin surrounding an abrasion. (INTERPRETATION: No restriction for acute application to the human skin.)

CATEGORY II - Compounds producing mild primary irritation of the intact skin and the skin surrounding an abrasion. (INTERPRETATION: Should be used only on human skin found by examination to have no abrasions or may be used as a clothing impregnant.)

CATEGORY III - Compounds producing moderate primary irritation of the intact skin and the skin surrounding an abrasion. (INTERPRETATION: Should not be used directly on the skin without a prophetic patch test having been conducted on humans to determine irritation potential to human skin. May be used without patch testing, with extreme caution, as clothing impregnants. Compound should be resubmitted in the form and at the intended use concentration so that its irritation potential can be reexamined using other test techniques on animals.

CATEGORY IV - Compounds producing moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion and, in addition, producing necrosis, vesiculation and/or eschars. (INTERPRETATION: Should be resubmitted for testing in the form and at the intended use concentration. Upon resubmission, its irritation potential will be reexamined using other test techniques on animals. prior to possible prophetic patch testing in humans, at concentrations which have been shown not to produce primary irritation in animals.)

CATEGORY V - Compounds impossible to classify because of staining of the skin or other masking effects owing to physical properties of the compound. (INTERPRETATION: Not suitable for use on humans.)

EYE CATEGORIES:

A. Compounds noninjurious to the eye. INTERPRETATION: Irritation of human eyes is not expected if the compound should accidentally get into the eyes, provided it is washed out as soon as possible.

B. Compounds producing mild injury to the cornea. INTERPRETATION: Should be used with caution around the eyes.

C. Compounds producing mild injury to the cornea, and in addition some injury to the conjunctiva. INTERPRETATION: Should be used with caution around the eyes and mucosa.

D. Compounds producing moderate injury to the cornea. INTERPRETATION: Should be used with extreme caution around the eyes.

E. Compounds producing moderate injury to the cornea, and in addition producing some injury to the conjunctiva. INTERPRETATION: Should be used with extreme caution around the eyes and mucosa.

F. Compounds producing severe injury to the cornea and to the conjunctiva. INTERPRETATION: Should be used with extreme caution. It is recommended that use be restricted to areas other than the face.

APPENDIX B

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Office certifies the following with regard to this study:

a. This study was conducted in accordance with:

(1) Standing Operating Procedures developed by the Toxicology Division, USAEHA, 1981.

(2) Title 21, Code of Federal Regulations (CFR), 1981 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

b. Facilities were inspected during its operational phase to insure compliance with paragraph 6.

c. The information presented in this report accurately reflects the raw data generated during the course of conducting the study.

PAUL V. SNEERINGER, Ph.D.
Chief, Analytical Quality
Assurance Office

DEPARTMENT OF THE ARMY
US ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP March 1981

STANDING OPERATING PROCEDURE

ORAL APPROXIMATE LETHAL DOSE (ALD) PROCEDURE

1. REFERENCES.

a. Title 21, Code of Federal Regulations (CFR), 1980 ed., Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

b. Guide for the Care and Use of Laboratory Animals, DHEW, NIH No. 78-23.

c. Standing Operating Procedure (SOP), HSE-LT/WP, this Agency, subject: Animal Facilities.

d. SOP, HSE-LT/WP, this Agency, subject: Individual Animal Identification.

2. PURPOSE. The purpose of the ALD procedure is to determine the minimum lethal dose of a compound using a small number of animals. This procedure lays the groundwork for the eventual determination of an LD_{50}. Except for the dosing procedure, this SOP is applicable to dermal and intraperitoneal ALD's.

3. BACKGROUND.

a. A range-finding procedure based on the work of Deichman and LeBlanc* is used to approximate the LD50. An ALD can be performed with a few animals in a short time.

b. All compounds will be handled with caution. Eye protection and rubber gloves will be worn at all times.

c. Disposable syringes will be destroyed in the syringe grinder in room 3202.

4. ANIMAL USE. The protocols for use of animals must be approved in advance by the Animal Use Review Committee, USAEHA. All animals will be cared for and handled according to the "Guide for the Care and Use of Laboratory Animals," (reference 1b) and the Toxicology Division SOP on animal facilities (reference 1c).

5. QUALITY ASSURANCE.

a. All test compounds will be characterized by infrared spectroscopy or other appropriate procedure for identification, purity, contaminants, and stability by the Environmental Chemistry Division, USAEHA, who will record the results according to the Good Laboratory Practices (reference 1a) and provide a copy to the Toxicology Division.

b. This SOP has been reviewed and approved by the USAEHA Quality Assurance Unit. The Quality Assurance Unit inspects a repeated test such as this one approximately once per month to assure that no significant problems exist that are likely to affect the integrity of the test.

6. METHOD. Dosages are calculated on the basis of each dosage being 50 percent higher than the dosage below it. Technical grade compound is preferred. See Appendix B for some doses and dosages. It has been found that the ALD is nominally approximately 30 percent higher than the LD50 of the same route in many cases.

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* Deichman, William B. and T. J. LeBlanc, Determination of the approximate lethal dose with about six animals, J Ind Hyg and Tox (25) 9: 415-417, November 1943. A reprint of this article is attached as Appendix A.

7. PROCEDURE FOR ORAL DOSING.

a. Select young adult rats; 200 g $\pm$ 25 g for males and 190 g $\pm$ 25 g for females. Mark them individually several days before dosing using the Toxicology Division marking system (reference 1d). Remove food 4 hours prior to dosing.

b. All rats will be dosed with the technical grade compound, if possible. If a solution must be made, the solvent chosen should have little toxicity of its own. Discuss solvent system with study director before use.

c. A single rat is dosed at each dose level. The rat is weighed and its dosage calculated. The amount delivered is based on the weight of the rat, the desired dose, and the density of the compound. For technical grade compounds a specific gravity of 1 (density = 1000 mg/mL) is assumed, unless known to be otherwise.

$$\text{Dosage (mL)} = \frac{\text{desired dose (mg/kg) x weight of rat (kg)}}{\text{density of solution}}$$

Because of limitations of measurement and delivery at the lower limit, the minimum volume delivered should not be less than 0.1 mL. The maximum volume delivered should not be greater than 0.01 mL/g body weight or 2.25 mL for a 225-g rat.

d. A curved oral dosing needle, about 2-3 inches long, 16 gauge with a ball tip approximately 3 mm in diameter, is used to dose the rats. They are available from Popper and Sons, Inc., 300 Denton Avenue, New Hyde Park, NY 11040, stock No. 7915, for 2 inch and stock No. 7916 for 3 inch.

e. Draw a volume greater than the dosage into a syringe that has a dosing needle attached. Invert the syringe and tap it to move any air bubbles to the top. Push all the air out of the syringe and dosing needle. Push excess liquid back into solution container. Dosage is now measured and in syringe.

f. Grasp rat from the back with the left hand so that the middle and forefinger are on the left and right sides of the rat's neck. The thumb secures the thorax caudal to the rat's right forelimb. The ring and little finger do the same on the left side.

g. With the right hand place the tip of the dosing needle near the back of the rat's mouth. Without forcing the syringe, allow the rat to chew and swallow the needle. When the needle is in the stomach, deliver the dosage and withdraw the syringe. One needle can be used for all dosing.

h. Return the rat to its cage and record the time of dosing on an ALD data sheet (see Appendix C). Observe the rat for any toxic signs (see Appendix D) and note the time of onset, severity, and duration. Rats will be observed each day until reversible toxic signs subside and every 3-4 days thereafter until the end of the study. The study is terminated when all signs of reversible toxicity subside or after 14 days, whichever occurs later. All rats will be grossly necropsied.

i. The ALD is the lowest dose which is lethal where two successively higher doses are lethal and the three doses lower are not lethal.

Example:	3333 mg/kg	dead
	2222 mg/kg	dead
	1480 mg/kg	dead
	987 mg/kg	alive
	658 mg/kg	alive
	439 mg/kg	alive

ALD = 1480 mg/kg.

PREPARED BY:

ARTHUR ASAKI
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APPROVED:

ARTHUR H. McCREESH, Ph.D.
Chief, Toxicology Division

CONRAD R. POPE, DVM
LTC, VC
Chairman, Animal Use Review Committee

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Auditor, Quality Assurance Unit

APPENDIX A

...TERMINATION OF THE APPROXIMATE LETHAL DOSE WITH ABOUT SIX ANIMALS*

WM. B. DEICHMANN[1] AND T. J. LEBLANC[2]

From the Kettering Laboratory of Applied Physiology and the Department of Preventive Medicine, College of Medicine, University of Cincinnati, Cincinnati, Ohio

IT IS frequently desirable to know the general order of the toxicity of a chemical compound used or proposed for use industrially. In many instances a highly accurate determination of the lethal dose for several species of experimental animals is required; at times a knowledge of the *Approximate Lethal Dose* is sufficient. With the method here reported it is possible to determine within broad limits the approximate lethal dose by using only about six animals.

Gaddum (1) in 1933 suggested a similar procedure to estimate the potency of an unknown preparation by administering a series of doses, each to a single animal, after the LD_{50} (the mean of the smallest effective and the largest ineffective dose) had been determined on a similar preparation. The progressive doses suggested by Gaddum are equal to m, $m \pm \lambda$, $m \pm 2\lambda$, etc., where m is the log of the LD_{50} and λ the standard deviation of th...garithms of the individual lethal doses. Ga... criterion of his own procedure applies equa...o the determination of the *Approximate Lethal Dose* suggested in this paper: "Such a test," (administration of a series of doses, each to a single animal) "is now known to be subject to very large errors owing to the variation between individual animals, but a large number of valuable results have been obtained by this simple technique, which is accurate enough for many purposes. Further, with solutions of which the potency is quite unknown any accurate test must be preceded by an approximate test made with single animals."

In the procedure reported here, graduated (staged) concentrations are employed, each one 50 per cent higher than the preceding one. The doses are 50 per cent progressions of 0.001 (Table 1A) and may be translated into any unit of measure the investigator chooses (grams, milligrams, milliliters, etc.). Doses are spaced sufficiently to preclude, practically, the possibility of killing an animal with one dose, while failing to kill with the next higher dose. The intervals between the doses are small enough, on the other hand, to result in a satisfactorily accurate determination of toxicity.

TABLE 1

STAGED CONCENTRATIONS FOR USE IN THE DETERMINATION OF THE APPROXIMATE LETHAL DOSE

A GRADUATED CONCENTRATIONS, EACH INCREASED BY APPROXIMATELY 50%, TO BE EMPLOYED IN DETERMINING LETHAL DOSES	B CALCULATED VALUES, EACH INCREASED BY 50% AND ACCURATE TO THE 4TH PLACE, ON WHICH CONCENTRATIONS IN A ARE BASED
0.0010	(0.0010)
0.0015	(0.0015)
0.0022	(0.0022)
0.0033	(0.0033)
0.005	(0.0049)
0.007	(0.0073)
0.010	(0.0109)
0.016	(0.0163)
0.024	(0.0244)
0.037	(0.0366)
0.055	(0.0549)
0.08	(0.0823)
0.12	(0.1234)
0.18	(0.1851)
0.28	(0.2776)
0.42	(0.4164)
0.62	(0.6246)
0.94	(0.9369)
1.4	(1.4053)
2.1	(2.1079)
3.2	(3.1618)
4.7	(4.7427)
7.1	(7.1140)
10.7	(10.6710)
16.0	(16.0065)
24.0	(24.0097)
36.0	(36.0145)

* Received for publication August 30, 1943.
[1] Kettering Laboratory of Applied Physiology.
[2] Department of Preventive Medicine.

HSE-LT/WP
SOP, Oral Approximate Lethal Dose (ALD) Procedure

In testing this method in 20 series of experiments, we found that in every case all concentrations up to a certain level resulted in survival of the animals, while above this level all concentrations killed. Series of experiments were also carried out with dosages spaced so as to follow a 40 per cent progression. Since the intervals between these doses are smaller, one might expect to be able to establish a more accurate lethal level by their use. Actually, however, animals survived doses that were higher than the lowest fatal dose, in two of the six series. From this it would appear that for practical purposes, bearing in mind that this is a method for approximations, a 40 per cent increment is too small and a 50 per cent increment seems to give satisfactory results within the limits of this experiment, hence any increase of the increment over 50 per cent would seem to be inadvisable and even unnecessary.

In Table 2 the *Approximate Lethal Dose* as determined by this method for a number of organic compounds is compared with the LD_{50} determined for each of them with the use of 60–90 animals and calculated by the method of maximum likelihood of Bliss (2). From this table it may be seen that the lowest killing concentrations, representing the approximate lethal doses, agree with the calculated LD_{50}'s within the limits of + 33 per cent and − 22 per cent.

TABLE 2

COMPARISON OF THE LD_{50}'S (DETERMINED FOR EACH COMPOUND ON 60-90 ANIMALS AND CALCULATED BY THE METHOD OF BLISS) WITH THE APPROXIMATE LETHAL DOSES OBTAINED BY THE METHOD HERE DESCRIBED

COMPOUND	SPECIES	MODE OF TREATMENT	SMALLEST LETHAL DOSE WHEN ONE ANIMAL IS TREATED WITH EACH DOSE SELECTED FROM TABLE 1A	LD_{50}	APPROXIMATE LETHAL DOSE EXPRESSED AS PER CENT OF LD_{50}	DEVIATION OF APPROXIMATE LETHAL DOSE FROM LD_{50}
			ml or gm/kg		*per cent*	*per cent*
Iron Carbonyl	Rabbit	Intravenous	0.01 ml	0.011	91	−9
Iron Carbonyl	Rabbit	Oral	0.016 ml	0.012	133	+33
Iron Carbonyl	Guinea Pig	Oral	0.024 ml	0.022	109	+9
Pentachlorophenol in Fuel Oil	Rat	Oral	0.024 gm	0.026	92	−8
Pentachlorophenol in Olive Oil	Rat	Oral	0.08 gm	0.078	103	+3
Na Pentachlorophenate in Water	Rat	Oral	0.18 gm	0.21	86	−14
Iron Carbonyl	Rabbit	Cutaneous	0.28 ml	0.24	117	+17
Methylcyclohexanol	Rat	Oral	1.4 gm	1.66	84	−16
Cyclohexanone	Rat	Oral	1.4 gm	1.84	76	+24
Cyclohexanone	Rat	Subcutaneous	2.1 gm	2.17	97	−3
Methylcyclohexanol	Rat	Subcutaneous	3.2 gm	2.90	110	+10
o-Nitrodiphenyl	Rabbit	Oral	4.7 gm	4.12	114	+14
p-Nitrodiphenyl	Rabbit	Oral	4.7 gm	4.44	106	+6
Methyl Methacrylate	Rat	Oral	10.0 ml	8.56	117	+17
Glycerol	Rat	Subcutaneous	16.0 ml	13.53	118	+18
Ethyl Methacrylate	Rat	Oral	16.0 ml	14.71	109	+9
Kerosene	Guinea Pig	Oral	16.0 ml	20.38	78	−22
Glycerol	Rat	Oral	24.0 ml	21.93	109	+9
Kerosene	Rabbit	Oral	24.0 ml	28.35	85	−15
Cyclohexane	Rat	Oral	36.0 gm	29.82	121	+21

METHOD

When beginning work with a new compound, the investigator can often make a rough estimate of the range of its probable toxicity, from the chemical formula, physical properties, and the apparent relationship of the compound to other familiar substances. On the basis of this estimate he selects about 6 consecutive doses (theoretically, only 2

a [illegible]uired) and treats a separate animal with each of these concentrations. In all likelihood, his results will be decisive, i.e., all animals treated with doses up to a certain level will survive while all those treated with higher doses will die. The *Approximate Lethal Dose* is the lowest concentration that kills. After the investigator has selected the range (from Table 1A) he wishes to use, he may, if he prefers, employ only four doses (and four animals), using every other dose over the range chosen. When these results have been obtained, the dose between the lowest lethal and the highest non-lethal dose may be tested, with one additional animal, for the final result.

SUMMARY

1. A method is presented whereby the *Approximate Lethal Dose* (or any other dose associated with a well defined effect) may be determined with the use of about six animals. A list of concentrations, representing a 50 per cent progression starting with 0.001, has been compiled and is given in Table 1A. These concentrations may be translated into any unit of measure the investigator chooses. The *Approximate Lethal Dose* is the lowest concentration that kills and may be determined by selecting about 6 consecutive concentrations and exposing one animal to each, or by selecting about 4 doses (and 4 animals) using every other dose over the range chosen; in this case one additional animal must be used to obtain the final result; this last animal is treated with the dose between the lowest lethal and the highest non-lethal concentration.

2. The *Approximate Lethal Dose* was determined for twenty organic compounds by experiments in which various species were used, and various modes of administration. The doses found agreed with the calculated LD_{50}'s (determined by the use of a large number of animals) within the limits of approximately ± 30 per cent.

REFERENCES

(1) GADDUM, J. H.: Methods of biological assay depending on a quantal response. Medical Research Council Reports, No. 138, 2, 1933.

(2) BLISS, C. I.: Determination of the small dosage mortality curve from small numbers. Quart. J. and Year Book of Phar., *11:* 192, 1938.

APPENDIX B

TABLE. DOSAGE (mL) FOR 200-g RAT

Dose (mg/kg)	Concentrations of Solutions						
	1,000 mg/mL	500 mg/mL	250 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	2 mg/mL
11,250	2.25						
7,500	1.50						
5,000	1.00	2.00					
3,333	0.67	1.33	2.67				
2,222	0.44	0.89	1.78				
1,480	0.30	0.59	1.19	2.96			
987	0.20	0.40	0.79	1.98			
658	0.13	0.26	0.53	1.32	2.63		
439	0.09	0.18	0.35	0.88	1.76	3.51	
293		0.12	0.23	0.59	1.17	2.34	
195		0.08	0.16	0.39	0.78	1.56	
130			0.10	0.26	0.52	1.04	
87			0.07	0.17	0.35	0.69	
58				0.12	0.23	0.46	
38				0.08	0.15	0.31	
26					0.10	0.21	2.57
17					0.07	0.14	1.71
11						0.09	1.14
8							0.76
5							0.51
3							0.34
2							0.23
1.5							0.15
1							0.10

APPENDIX C

SINGLE DOSE ADMINISTRATION (Approximation) (Assay) (Symptomatology)

Project No._____ ENT No._____

Compound Name_____

Mode of Administration_____ Species_____ Sex_____

Diluent_____ Technician_____ Date_____

Time Food Removed_____ Type of Food_____

Date Animal Born_____ Dose_____Mg/K Concentration of Compound (W/V)_____

Number Cage Ani.	Wt (gm) (kg)	Vol Del cc	Time (on 24 hr. basis) Adm	Effect	min	Recover	min	Dead	min	Symptoms
Average										

Mortality	24 hours	48 hours	72 hours	
	/	/	/	

Comments: Detailed description of dose preparation and administration (over)

APPENDIX B

TABLE. DOSAGE (mL) FOR 200-g RAT

Dose (mg/kg)	Concentrations of Solutions						
	1,000 mg/mL	500 mg/mL	250 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	2 mg/mL
11,250	2.25						
7,500	1.50						
5,000	1.00	2.00					
3,333	0.67	1.33	2.67				
2,222	0.44	0.89	1.78				
1,480	0.30	0.59	1.19	2.96			
987	0.20	0.40	0.79	1.98			
658	0.13	0.26	0.53	1.32	2.63		
439	0.09	0.18	0.35	0.88	1.76	3.51	
293		0.12	0.23	0.59	1.17	2.34	
195		0.08	0.16	0.39	0.78	1.56	
130			0.10	0.26	0.52	1.04	
87			0.07	0.17	0.35	0.69	
58				0.12	0.23	0.46	
38				0.08	0.15	0.31	
26					0.10	0.21	2.57
17					0.07	0.14	1.71
11						0.09	1.14
8							0.76
5							0.51
3							0.34
2							0.23
1.5							0.15
1							0.10

APPENDIX D

TABLE. SOME TOXIC SIGNS TO OBSERVE

Neuromuscular-Skeletal	Ataxia Paralysis Prostration Catalepsy Muscle tone Loss of consciousness	Tremors Fasciculations Clonic convulsions Tonic convulsions Death	Increased or decreased sensitivity to pain, sound, touch Opisthotonus Emprosthotonus
Behavioral	Sedation-hypoactivity Restlessness-hyperactivity Drooping head Depression Excessive preening	Irritability Hostility Gnawing Posture, tail, hunch Unusual movements	Increased or unusual vocalization Abnormal gait
Respiratory	Hypopnea (usually shallow breaths at normal rate) Hyperpnea (deep, rapid breaths)	Apnea (periodic pauses in breathing) Dyspnea (shallow, rapid breaths) Gasping Wheezing	
Gastrointestinal-Genitourinary	Salivation Rhinorrhea (discharge from nose) Retching	Hematuria (bloody urine) Constipation Diarrhea Bloody stool	
Eyes	Miosis (contraction of of pupils) Mydriasis (dilation of of pupils) Glassy eyed stare (no blinking) Ptosis (drooping eyelid) Exophthalmus (bug eye)	Nystagmus (rhythmical oscillation of eyeball) Lacrimation (tears) Pupillary light reflex (pupils contract when light is shined into eyes) Corneal reflex (blink when cornea touched)	
Circulation	Cyanosis Palor Flushed Hemorrhage		
Local Tissue Irritation	Edema Erythema Necrosis Ischemia (local palor without swelling)		
General Health	Dehydration Scoliosis (bent spine) Swollen joints Bent bones		

DEPARTMENT OF THE ARMY
US ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MD 21010

HSE-LT/WP

January 1981

STANDING OPERATING PROCEDURE

PRIMARY EYE IRRITATION STUDY

Paragraph

Appendices

1. PURPOSE. To determine the irritative potential of the test article to the eyes of New Zealand White Rabbits following one application.

2. SCOPE. This standing operating procedure (SOP) is compiled for use in the animal facilities of the Toxicology Division, US Army Environmental Hygiene Agency (USAEHA) and is to be endorsed and periodically revised by the Animal Use Review Committee, USAEHA; the Analytical Quality Assurance Office, USAEHA; and approved by the Chief, Toxicology Division.

3. REFERENCES. See Appendix A.

4. ANIMAL CARE AND SELECTION.

a. Special attention will be given to proper and humane treatment of all laboratory animals in accordance with the "Guide for the Care and Use of Laboratory Animals."

b. Testing shall be performed on healthy, young New Zealand White Albino Rabbits.

c. Caging shall be designed to minimize exposure to sawdust, wood chips, and other extraneous materials that might enter the eye.

d. Water and food shall be provided ad libitum.

5. STUDY DESIGN.

a. Condition of Test Substance.

(1) If the test substance is a liquid, it must be placed in the eye undiluted.

(2) If the test substance is a solid or granular product, it must be ground into a fine dust or powder. The test substance must not be moistened before it is placed in the eye.

b. Condition of Animals.

(1) The eyes must be examined using fluorescein dye procedures at least 24 hours before application of the test substance.

(2) Animals showing preexisting corneal injury are to be eliminated.

c. Number of Animals. At least nine animals must be used.

d. Number and Selection of Dose.

(1) A dose of 0.1 ml of liquid or 100 mg of solid must normally be applied to each test eye.

(2) Smaller quantities may be used when the standard quantities would be lethal or when 100 mg of the solid cannot feasibly be administered to the eye.

6. STUDY CONDUCT.

a. The test substance must be placed on the everted lower lid of one eye; the upper and lower lids are then to be gently held together for 1 second before releasing to prevent loss of material. The other eye, remaining untreated, serves as a control.

b. The treated eyes of six rabbits must remain unwashed. The remaining three rabbits receive test material, and the treated eye is flushed for 1 minute with lukewarm tap water starting no sooner than 20-30 seconds after instillation.

c. A local anesthetic to reduce pain in test animals may be used prior to administration of the test substance, provided that evidence can be presented indicating no significant difference in toxic reaction to the test substance will result from use of the anesthetic.

7. OBSERVATION AND SCORING.

a. Readings of ocular lesions must be made at 24, 48, and 72 hours after treatment. Readings must be made every 3 days thereafter if injury persists for at least 13 days after treatment or until all signs of reversible toxicity subside.

b. Grading and scoring of irritation are to be performed in accordance with Table 1. The most serious effects, such as pannus or blistering of the conjunctivae and other effects indicative of corrosive action must be reported separately.

TABLE 1. SCALE FOR SCORING OCULAR LESIONS.

---

1. Cornea

a. Opacity-degree of density (most dense area taken for reading)
No opacity.....0
Scattered or diffuse area, details of iris clearly visible.....1
Easily discernible translucent areas, details of iris slightly obscured.....2
Opalescent areas, no details of iris visible, size of pupil barely discernible.....3
Opaque, iris invisible.....4

b. Area of cornea involved
One quarter (or less) but not zero.....1
Greater than one quarter but less than one half.....2
Greater than one half but less than three quarters.....3
Greater than three quarters up to whole area.....4

Score = (a) x (b) x (5) = Total max score = 80

2. Iris

a. Values
Normal.....0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive).....1
No reaction to light, hemorrhage, gross destruction (any or all of these) 2

Score = (a) x 5 Total max score = 10

3. Conjunctivae

a. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
Vessels normal.....0
Vessels definitely injected above normal.....1
More diffuse, deeper crimson red, individual vessels not easily discernible.....2
Diffuse beefy red.....3

b. Chemosis

No swelling.....0
Any swelling above normal (included nictitating membrane).....1
Obvious swelling with partial eversion of lids.....2
Swelling with lids about half closed.....3
Swelling with lids about half closed to completely closed.....4

c. Discharge

No discharge.....0
Any amount different from normal (does not include small amounts observed in inner cnathus of normal animals.....1
Discharge with moistening of the lids and hairs just adjacent to lids.....2
Discharge with moistening of the lids and hairs, and considerable area around the eye.....3

Score (a + b + c) x 2 Total max score = 20

The individual numerical scores for each eye to which a given compound has been applied are added together and then divided by the number of eyes used to obtain the score. Results are recorded on HSE-LT Form 51, Primary Eye Iritation, Rabbit Eye Chart (Appendix B); and calculations are shown on HSE-LT Form 48, Acute Eye Irritation - Rabbits (Appendix C).

c. For reporting convenience, the following eye injury categories are established and defined in Table 2.

TABLE 2. EYE INJURY CATEGORIES.

---

1. CATEGORY A - Compounds noninjurious to the eye
   Eye injury score limits: 0-10 (individual conjunctival score for chemosis, redness or discharge not to exceed 1).
   Interpretation - Irritation of human eyes is not expected if the compound should accidentally get into the eyes, provided it is washed out as soon as possible.

2. CATEGORY B - Compounds producing mild injury to the cornea.
   Eye injury score limits: 10-20 (individual conjunctival score for chemosis, redness or discharge not to exceed 1).
   Interpretation - To be used with caution around the eyes.

3. CATEGORY C - Compounds producing mild injury to the cornea and, in addition, some injury to the conjunctiva. Eye injury score limits: 5-30 (individual conjunctival score for chemosis, redness, or discharge exceed 1).
Interpretation - To be used with caution around the eyes and mucosa (e.g., nose and mouth). Eye injury score limits: 5-30

4. CATEGORY D - Compounds producing moderate injury to the cornea.
Eye injury score limits: <20-50 (individual conjunctival score for chemosis, redness, or discharge not to exceed 1).
Interpretation - To be used with extreme caution around the eyes. Keep away from ocular area.

5. CATEGORY E - Compounds producing moderate injury to the cornea and, in addition, producing some injury to the conjunctiva. Eye injury score limits: 20-50 (individual conjunctival score for chemosis, redness, or discharge exceed 1).
Eye injury score limits: 20-50
Interpretation - To be used with extreme caution around the eyes and mucosa (e.g., nose and mouth). Keep away from ocular areas.

6. CATEGORY F - Compounds producing severe injury to the cornea and conjunctiva.
Eye injury score limits: 50 or greater.
Interpretation - To be used with extreme caution, recommended that use be restricted to areas other than the face.

---

8. REPORTING.

a. HSE-LT Forms 48 and 51 are to be completed, signed, dated, and placed into the appropriate project number file in the Toxicology Division's Preventative Medicine Reference-Active Project File.

b. An eye injury category is assigned using Table II as a guide, and this is recorded in Laboratory Notebook 10, Topical Hazard Evaluation Program.

c. The eye injury category, with explanation and a copy of HSE-LT Form 39-1, Acute Eye Effects - New Zealand White Rabbits (Appendix D) is to be included in the Topical Hazard Evaluation Program Report.

9. APPROVALS.

a. This study will be run in accordance with Good Laboratory Practices (21 CFR 58) and approved by the Animal Use Review Committee.

CONRAD R. POPE, DVM
LTC, VC
Chairman, Animal Use
Review Committee

b. This SOP has been reviewed and approved by the USAEHA Quality Assurance Office. The Quality Assurance Office inspects an in-process procedure of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of procedure.

PAUL V. SNEERINGER, Ph.D.
Chief, Analytical Quality
Assurance Office

c. Designated Toxicology Division personnel will be responsible for the performance of this primary eye irritation study SOP.

ARTHUR H. McCREESH, Ph.D.
Chief, Toxicology Division

d. This primary eye irritation study SOP was prepared by.

MICHAEL J. TOPPER, DVM
CPT, VC
General Veterinary Officer
Toxicology Division

APPENDIX A

REFERENCES

1. Title 21, Code of Federal Regulations (CFR), 1979 ed., Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

2. Proposed Rules, Health Effects Test Standards for Toxic Substances Control Act Test Rules and Proposed Good Laboratory Practice Standards for Health Effects, 44 Federal Register (FR) 44054, 26 July 1979.

3. Guidebook: Toxic Substances Control Act, Vol I, 1977.

4. Draize, J. H., G. Woodard, and H. O. Calvery, Methods for the Study of Irritation and Toxicitiy of Substances Applied Topically to the Skin and Mucous Membranes. J Pharmacol Exp Ther, 83:377-390, 1944.

5. Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics-Dermal Toxicity, pp 49-52. Assoc of Food and Drug Officials of the U.S., Topeka, Kansas, 1965.

6. Guide for the Care and Use of Laboratory Animals, DHEW, NIH, No. 78-23 (revised, 1978).

APPENDIX B

PRIMARY EYE IRRITATION

RABBIT EYE CHART

PROJECT #_____ CHEMICAL NAME:_____

ENT #_____ _____

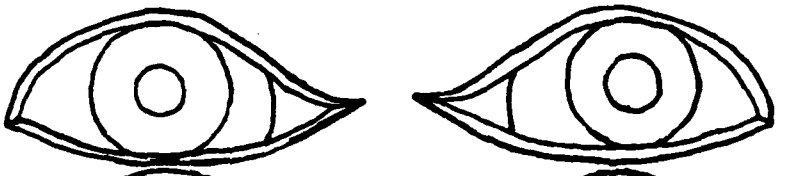
DATE STARTED:_____ PHYSICAL STATE:_____

TECHNICIAN_____ AMOUNT APPLIED:_____

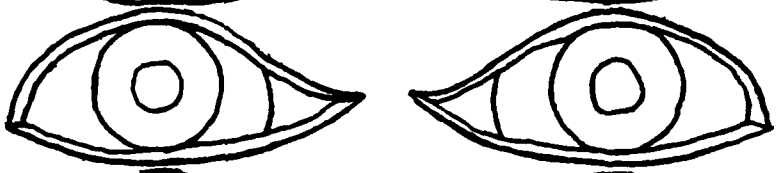
RABBIT NUMBER

Right Eye - test Left eye - control

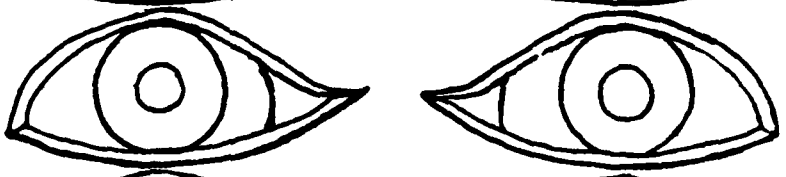
Pre-test



24-hour

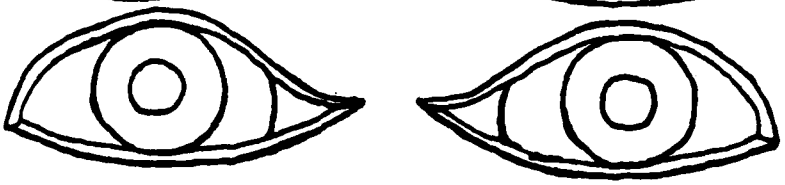


48-hour



72-hour

7-day



REMARKS: Pre-test
24-hour
48-hour
72-hour
7 day

HSE-LT Form 51, 1 Jun 80

APPENDIX C

ACUTE EYE IRRITATION

RABBITS

PROJECT #_____ CHEMICAL NAME:_____

ENT #_____ _____

DATE STARTED:_____ PHYSICAL STATE_____

TECHNICIAN:_____ AMOUNT APPLIED:_____

		RABBIT NUMBER					
TISSUE	EFFECT	1	2	3	4	5	SCORE
Cornea	A. Opacity						
	B. Amount Area Involved						
	Score = (AXBX5)						= Subtotal
Iris	A. Iritis						
	Score = (AX5)						= Subtotal
Conjunctiva	A. Redness						
	B. Chemosis						
	C. Discharge						
	Score = (A+B+C)X2						= Subtotal

TOTAL IRRITATION SCORE =

1. Ref: "Primary Irritation Evaluation Program."
2. Evaluation: Eye Injury Score (Total Score/6eyes =_____

   Eye Injury Category =_____
3. Interpretation:
4. Remarks:

HSE-LT Form 48, 1 Jun 80

APPENDIX D

COMPOUND:

ACUTE EYE EFFECTS NEW ZEALAND WHITE RABBITS	TOXICITY CATEGORY	CONDITIONS -

Time of Reading		Scores Rabbit No.							
Hrs-Days	Structure	1	2	3	4	5	6	Mean Score	Comments
24	cornea iris conjunctivae								
48	cornea iris conjunctivae								
72	cornea iris conjunctivae								
7-days	cornea iris conjunctivae								

HSE-LT Form 39-1, 1 Jun 80

DEPARTMENT OF THE ARMY
US ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP

STANDING OPERATING PROCEDURE

PRIMARY DERMAL IRRITATION STUDY

TOXICOLOGY DIVISION

1. PURPOSE. To determine the irritative potential of the test article to the skin of New Zealand White rabbits on one application.

2. SCOPE. This standing operating procedure (SOP) is compiled for use in the animal facilities of the Toxicology Division (HSE-LT), US Army Environmental Hygiene Agency (USAEHA) and is to be endorsed and periodically revised by the Animal Use Review Committee, USAEHA; the Analytical Quality Assurance Office, USAEHA; and approved by the Chief, Toxicology Division.

3. REFERENCES.

a. Title 21, Code of Federal Regulations (CFR), 1979 ed., Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

b. Proposed Rules, Primary Dermal Irritation Study, 44 Federal Register (FR) 44071, 26 July 1979.

c. Guidebook: Toxic Substance Control Act, Volume 1, 1977.

d. Draize, J. H., G. Woodard, and H. O. Calvery, Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membrane, J. Pharmacol Exp Ther, 83:377-390, 1944.

e. Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity, pp 49-52, Assoc of Food and Drug Officials of the US, Topeka, Kansas, 1965.

f. Guide for the Care and Use of Laboratory Animals, DHEW, NIH No. 78-23.

4. ANIMAL CARE AND SELECTION.

a. Special attention will be given to proper and humane treatment of all laboratory animals in accordance with the "Guide for the Care and Use of Laboratory Animals."

b. Testing shall be performed on healthy, young New Zealand White albino rabbits.

c. Water and food shall be provided ad libitum.

5. STUDY DESIGN.

a. Condition of Test Substance.

(1) If the test substance is a liquid, it must be applied undiluted.

(2) If the test substance is a solid, it must be slightly moistened with physiological saline before application.

b. Number of Animals. At least six (6) animals must be used.

c. Number and Selection of Dose. A dose of 0.5 mL of liquid or 0.5 g of solid or semisolid is to be applied to each application site.

d. Control Groups.

(1) A vehicle control group is required if the vehicle is known to cause any toxic dermal reactions or if there is insufficient information about the dermal effects of the vehicle.

(2) Separate animals are not required for an untreated control group. Each animal serves as its own control.

6. STUDY CONDUCT.

a. The application sites on the back of the animals must be clipped free of hair.

b. Two skin sites must be abraded with a 20- or 21-gauge needle so as to penetrate the stratum corneum but not the dermis.

c. The test substance is applied to three intact and three abraded skin sites.

d. The skin sites are covered with 2-inch by 2-inch gauze patches secured with adhesive tape.

e. A wrapping material made of an impervious, nonreactive material such as rubber or plastic is required to keep the test substance in contact with the skin.

f. The animals should be kept restrained for 24 hours.

g. At the end of 24 hours, the animal should be unwrapped and gauze removed. If any test substance is still remaining, the skin should be wiped off (but not washed).

7. OBSERVATION AND SCORING.

a. Animals must be observed and signs of erythema and edema must be scored at 24 hours and 72 hours after application of the test substance. The observation for irritation and scoring of any irritation must continue daily until all irritation subsides or is obviously irreversible.

b. Grading and scoring of irritation are to be performed in accordance with Tables 1 and 2. The most serious effects, such as severe edema, vesiculation, ulceration, or necrosis should be reported separately.

c. Results are recorded on HSE-LT Form 47 (Summary of Primary Skin Irritation Test), Appendix A.

d. For reporting convenience, the following skin injury categories are established and defined in Table 2.

TABLE 1. SCALE FOR SCORING SKIN REACTIONS

1.	ERYTHEMA AND ESCHAR FORMATION.	
	a. No erythema	0
	b. Very slight erythema (barely perceptible)	1
	c. Well defined erythema	2
	d. Moderate-to-severe erythema	3
	e. Severe erythema ("beet" redness to slight eschar formation injurious in depth)	4
	f. Possible total erythema score	4*
2.	EDEMA FORMATION.	
	a. No edema	0
	b. Very slight edema (barely perceptible)	1
	c. Slight edema (edges of area well defined by definite raising)	2
	d. Moderate edema (edges raised approximately 1 mm)	3
	e. Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
	f. Possible total edema score	4*
3.	POSSIBLE TOTAL SCORE FOR PRIMARY IRRITATION.	8

* Any skin reaction more serious than severe edema, vesiculation, ulceration, or necrosis places the chemical in category V.

TABLE 2. SKIN INJURY CATEGORIES

1. CATEGORY I. Compounds producing no primary irritation of the intact skin or no greater than mild primary irritation of the skin surrounding an abrasion.

a. Interpretation. No restriction for acute application to the human skin.

b. Score Limits. Intact 0-0.5 Abraded 0.51-2.0 Total 0-2.0

2. CATEGORY II. Compounds producing mild primary irritation of the intact skin and the skin surrounding an abrasion.

a. Interpretation. Should be used only on human skin found by examination to have no abrasions or may be used as a clothing impregnant.

b. Score Limits. Total 0.51-2.0 Intact > 0.5.

3. CATEGORY III. Compounds producing moderate primary irritation of the intact skin and the skin surrounding an abrasion.

a. Interpretation. Should not be used directly on the skin without a prophetic patch test having been conducted on humans to determine irritation potential to human skin. May be used without patch testing, with extreme caution, as clothing impregnants. Compound should be resubmitted in the form and at the intended use concentration so that its irritation potential can be reexamined using other test techniques on animals.

b. Score Limits. Total 2.1-5.0

4. CATEGORY IV. Compounds producing moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion and, in addition, producing necrosis, vesiculation and/or eschars.

a. Intrepretation. Should be resubmitted for testing in the form and at the intended use concentration. Upon resubmission, its irritation potential will be reexamined using other test techniques on animals, prior to possible prophetic patch testing on humans at concentrations which have been shown not to produce primary irritation in animals.

b. Score Limits. Total 2.1-7.9

5. CATEGORY V. Compounds impossible to classify because of staining of the skin or other masking effects owing to physical properties of the compound.

a. Interpretation. Not suitable for use on humans.

b. Score Limits. Total 8.0

8. REPORTING.

a. HSE-LT Form 47 is to be completed, signed, dated, and placed into the appropriate project number file in the Toxicology Division's Preventive Medicine Reference - Active Project File.

b. A skin injury category is assigned using Table 2 as a guide and this is recorded in laboratory notebook 10 (Topical Hazard Evaluation Program).

c. The skin injury category, with explanation and a copy of HSE-LT Form 39-2 (Primary Skin Effects - New Zealand White Rabbits), Appendix B, is to be included in the Topical Hazard Evaluation Program Report.

9. APPROVALS.

a. This study will be run in accordance with Good Laboratory Practices (21 CFR 58) and has been reviewed and approved by the USAEHA Animal Use Review Committee.

CONRAD R. POPE, DVM
LTC, VC
Chairman, Animal Use Review
Committee

b. This study SOP has been reviewed and approved by the USAEHA Analytical Quality Assurance Office.

PAUL V. SNEERINGER, Ph.D.
Chief, Analytical Quality
Assurance Office

c. Designated Toxicology Division personnel will be responsible for the performance of this primary dermal irritation study SOP.

ARTHOR H. McCREESH, Ph.D.
Chief, Toxicology Division

d. This primary dermal irritation study SOP was prepared by:

MICHAEL J. TOPPER, DVM
CPT, VC
General Veterinary Office
Toxicology Division

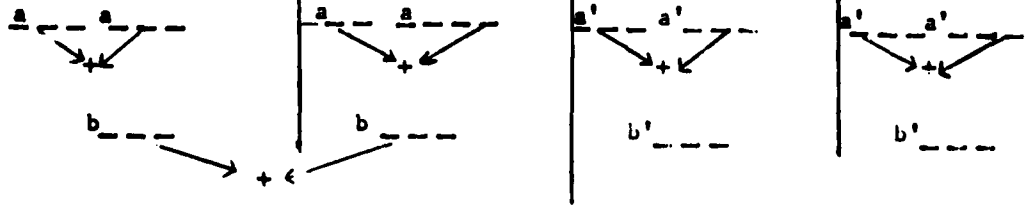
APPENDIX A

SUMMARY OF PRIMARY SKIN IRRITATION TEST

Study No._____ Chemical Name_____
ENT No._____ _____
Date Started_____ pH & Physical State_____
Technician_____ Concentration Tested_____
Amount Applied_____

IRRITATION SCORES

	INTACT SKIN SITES							ABRADED SKIN SITES						
Rabbit No.	Erythema			Edema				Erythema			Edema			
	24 hr	72 hr	7 day	24 hr	72 hr	7 day		24 hr	72 hr	7 day	24 hr	72 hr	7 day	



Intact Score = $\frac{C}{\text{No. of rabbits on test}}$ _____

Abraded Score = $\frac{C'}{\text{No. of rabbits on test}}$ _____

Total Score = $\frac{C + C'}{2 \times \text{No. of Rabbits on test}}$ _____

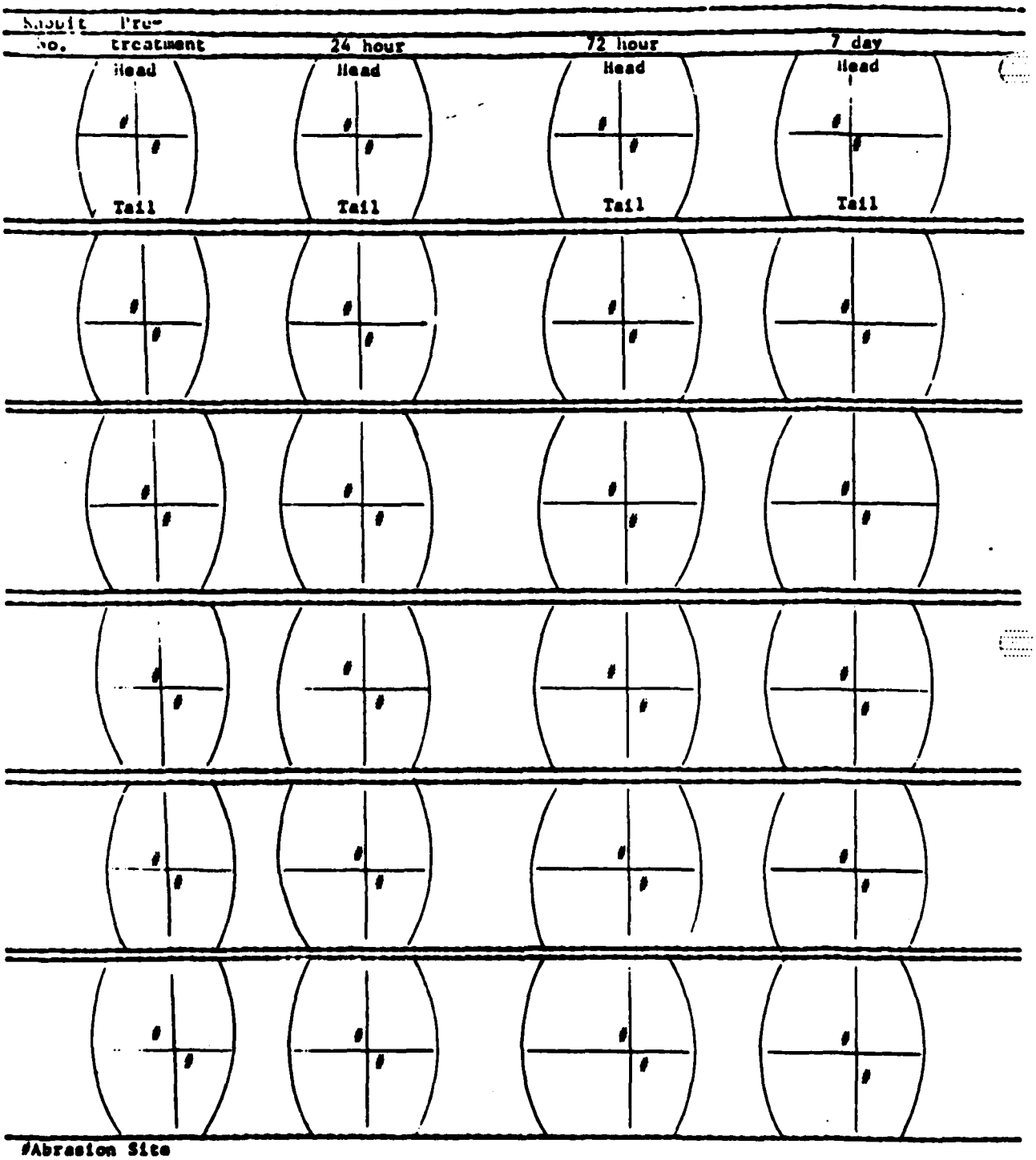
Primary Skin Irritation Index _____

REMARKS:_____

_____

HSE-LT Form 47, 1 Jun 80

HSE-LT/WP
SOP, Primary Dermal Irritation Study



APPENDIX B

COMPOUND:

PRIMARY SKIN EFFECTS
NEW ZEALAND WHITE RABBITS

TOXICITY CATEGORY

CONDITIONS -

	Time of Observation (Hours)	Response Rabbit No. 1	2	3	4	5	6	Mean Score	Comments
Erythema & Eschar									
Intact Skin	24								
Intact Skin	72								
Abraded Skin	24								
Abraded Skin	72								
								___	
	Subtotal							___	
Edema Formulation									
Intact Skin	24								
Intact Skin	72								
Abraded Skin	24								
Abraded Skin	72								
								___	
	Subtotal							___	
	Total							___	

HSE-LT Form 39-2, 1 Jun 80

DEPARTMENT OF THE ARMY
US ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP 20 November 1980

STANDING OPERATING PROCEDURE BASIC SAFETY ASSESSMENT TEST PROCEDURE FOR PRIMARY DERMAL PHOTOCHEMICAL SKIN IRRITATION STUDY IN RABBITS

1. PURPOSE. To determine the relative toxicity of a test article when it is placed on the skin as one dermal application and irradiated with UV light.

2. INTRODUCTION.

a. Photochemical skin reactions may be demonstrated when rabbit skin is exposed to ultraviolet (UV) radiation following topical administration of various chemicals.

b. The introduction of a chemical substance into a biologic system may cause a localized reaction on the skin following UV irradiation so that a photodynamic event is initiated, i.e. skin irritation.

c. These studies are performed to determine the phototoxic potential of a given chemical applied to rabbit skin and then irradiated by UV light. Individual chemicals or combination of chemicals in ethanol solutions are applied to rabbit skin and the irritation reactions are compared to a simultaneously applied known photochemical skin irritant (Bergamot oil).

d. All compounds are handled with caution. Current test procedures cannot eliminate the possibility of individual skin sensitivity to certain compounds. EYE PROTECTION AND GLOVES WILL BE WORN AT ALL TIMES. Chemicals tested for phototoxic skin reactions are graded according to their primary skin irritation reactions.

e. Compounds that produce no photochemical skin related reactions are considered not to be photochemical skin irritants within the limits of the present test protocol.

f. A test procedure based on the studies of Marzulli and Maibach (1970)* is employed to determine the phototoxic potential of candidate repellents.

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* Francis Marzulli and Howard I. Maibach, "Perfume Phototoxicity", J. Soc. Cosmet. Chem., 21, pp 695-715 (September 1970).

g. The study described in this SOP will be conducted according to the guidelines stated in "Guide for the Care and Use of Laboratory Animals, "US Department of Health, Education and Welfare Publication No. (NIH) 74-23, revised 1978.

3. METHODS.

a. Test Species. New Zealand White Albino rabbits. Six per test.

b. Sample Required. 0.5 gms or 0.5 mL.

c. Duration of Test. Three days for skin reactions. Seven days for final report. Ten days for total.

d. Procedure.

(1) Six animals (6 males or females) will be used. The backs of all animals will be shaved on the day before irradiation over an area of at least 130 sq cm of the body surface area.

(2) One line will be drawn down the mid-line of the animal's backs using a felt ink pen.

(3) Three test compounds each contained in 0.05 mL of 95 percent ethyl alcohol are applied on the back to the right of the mid-line of each rabbit. The test compounds are applied as 25 percent solutions (w/v) in 95 percent ethyl alcohol. One additional compound applied along with the test compounds is a 10 percent solution (w/v) of Bergamot oil† in 95 percent ethyl alcohol that serves as a positive control.

(4) The animals are immobilized in stainless steel restrainers during compound application and during UV irradiation. The compounds are applied to the rabbit's back in random order with at least 4 cm spacing between application sites. They are allowed to remain undisturbed for 5 minutes and then irradiated for 30 minutes with an UV lamp‡ held at distances of 10-15 cm from the application sites. The emission spectrum of the radiation source was measured using a EG&G spectroradiometer. Over 95 percent of the ultraviolet radiation output was 365 nm with an intensity of 600 u watts/cm^2.

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† Source: Oil Bergamot Italian, Ungerer & Company, 161 Avenue of the Americas, New York, NY 10013

‡ A "Spectroline" ultraviolet lamp (or equivalent) serves as the radiation source. The spectroline lamp is from the Black Light Eastern Corp., Westbury, L.I., NY, but is also available from Scientific Products, Washington, DC, as the Blak-Ray lamp, catalog item no. L6093. The emitted spectra from each lamp are charted by personnel of Laser Microwave Division at regular intervals of 6 months.

(5) Following irradiation, the UV light is removed and 5 minutes later the same volume of the four compounds are applied in the same order onto the left side of the rabbit. These sites serve as nonirradiated control areas, and are used to compare any inherent skin irritant properties of the compounds with that observed following UV irradiation. All skin sites are left unoccluded throughout the test procedure.

(6) All test chemicals are stored at room temperature in fume hoods.

4. SCORING.

a. The skin is observed at 24, 48, and 72 hours after application and the reactions produced by the compounds are evaluated on the basis of weighted scores (Table). The individual evaluation scores for the UV irradiated sites are added and divided by the number of observations to give a "total skin irritation score" (R_1). The score (R_2) for the nonirradiated sites is calculated as above and subtracted from the R_1 score to give a NET total photochemical skin irritation score.

TABLE. EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beet redness to slight eschar formation injurious in depth)	4
Possible total erythema score:	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (edges raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Possible total edema score	4
Possible total score for primary irritation	8

b. The individual erythema scores for the UV irradiated sites are added and divided by the number of observations (18) to give a "total UV skin erythema score" (e). The score (f) for edema is calculated in the same manner. The scores (g and h) for erythema and edema for the non UV sites are calculated as above and subtracted from their respective e and f scores to give NET photochemical skin erythema and edema scores.

c. A modified HSE-LT-T Form T-24 is used to summarize the skin irritation scores (Figure).

d. A photochemical toxic skin reaction is characterized by erythema and edema during the 72 hours following the irradiation. A test compound or formulation is considered to cause a photochemical skin irritation reaction when the final NET total score of erythema is greater than 1.0 and/or for edema 0.5 or greater.

5. NOTICE (GLP). This study will run in accordance with 21 CFR 58, Good Laboratory Practices, and as approved by Animal Use Review Committee.

Chairman, Animal Use Review Committee

6. QUALITY ASSURANCE (QA). This study SOP has been reviewed and approved by the USAEHA Quality Assurance Unit. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Auditor, Quality Assurance Unit

7. RESPONSIBILITY. Designated Toxicology Division personnel will be responsible for the performance of this photochemical SOP.

Chief, Toxicology Division

PREPARED BY:

MAURICE H. WEEKS
Chief, Toxicity Evaluation Branch
Toxicology Division

Summary of Photochemical Skin Irritation Test

Study No._____ Chemical Name_____

ENT No._____

Date Started_____ pH & Physical State_____

Technician_____ Concentration Tested_____

Amount Applied_____

IRRITATION SCORES

	UV Skin Sites						Non UV Skin Sites					
Rabbit No.	Erythema 24 hr	48 hr	72 hr	Edema 24 hr	48 hr	72 hr	Erythema 24 hr	48 hr	72 hr	Edema 24 hr	48 hr	72 hr
	a___	a___	a___	a___	a___	a___	a'___	a'___	a'___	a'___	a'___	a'___
		+			+			+			+	
		b___			c___			b'___			c'___	
				+						+		
				d___						d'___		

R_1 (total UV) = d/No. of Obs. (18) = _____ Erythema: e = b/18 _____ Edema: f = c/18_____

R_2 (total Non UV) = d'/No. of Obs. (18) = _____ g = b'/18_____ h = c'/18_____

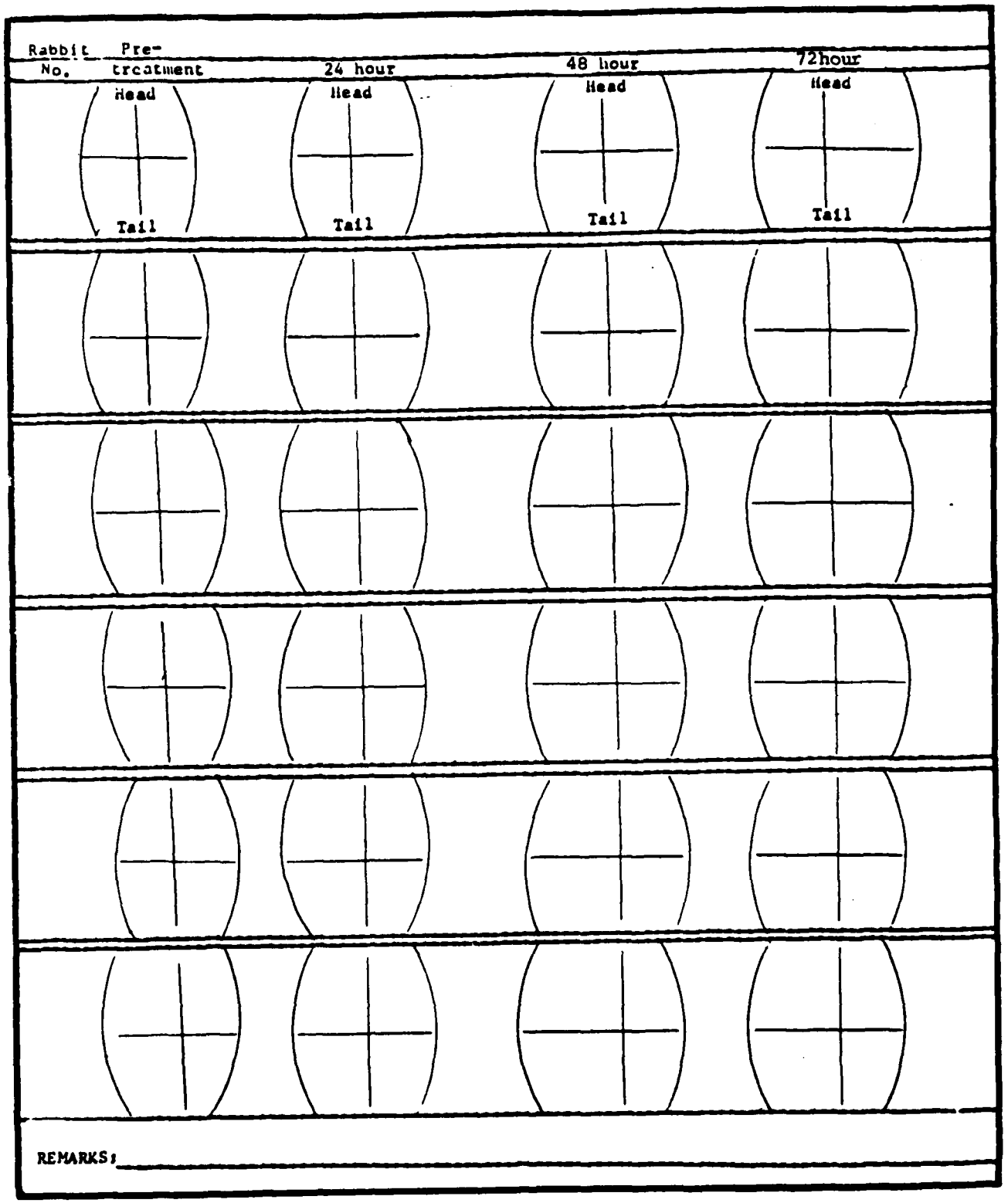
Net UV Score = R_1 - R_2 = _____ Net=_____ _____

REMARKS:_____

_____

HSE-LT Form 44, 1 Jun 80

Replaces USAEHA Form 115, 3 Dec 75, which will be used.


Rabbit No.
Pre-treatment
24 hour
48 hour
72hour
Head
Tail
REMARKS:

APPENDIX

PHOTOCHEMICAL IRRITATION-NEW ZEALAND WHITE RABBITS

COMPOUND:	USAEHA STUDY NO.
COMMENTS:	
PROCEDURE:	

	MEAN SKIN IRRITATION SCORE							
	Test Compound UV Exposure		Test Compound Non-UV Exposure		Positive Control UV Exposure		Positive Control Non-UV Exposure	
Observation Time	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
24 Hours								
48 Hours								
72 Hours								
TOTAL								
Mean Irritant Responses								
Net Score								

AEHA Form 62, 1 Feb 81 (HSE-LI)

DEPARTMENT OF THE ARMY
US ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MD 21010

HSE-LT/WP August 1981

STANDING OPERATING PROCEDURE

GUINEA PIG SKIN SENSITIZATION TEST

1. PURPOSE. To determine skin sensitization reaction of various chemicals in male Hartley strain albino guinea pigs.

2. INTRODUCTION.

a. Skin sensitization is a phenomenon wherein the response obtained by exposing the skin to a chemical over a prolonged period of time is significantly greater than that obtained from a single exposure.

b. All compounds are handled with caution. Current test procedures cannot eliminate the possibility of individual skin sensitization to certain chemicals. Eye protection and gloves will be worn at all times.

c. Compounds that produce no sensitization reactions will be a considered not to be a sensitizer within the limits of the present test protocol.

d. This test procedure is based on the studies of Landsteiner* and is used to predict possible skin sensitizations.

3. REFERENCES.

a. Title 21, Code of Federal Regulations (CFR), 1980 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

b. US Department of Health, Education, and Welfare (HEW) Publication No. 78-23, revised 1978. Purpose: Is the male Hartley strain albino guinea pig the only guinea pig that can be used for skin sensitization test?

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* The Landsteiner Guinea Pigs Sensitization Test, as modified by the Chemical Hygiene Fellowship, Mellon Institute, July 1967.

4. METHODS.

a. Tests Species. Male Hartley strain albino guinea pigs.

b. Sample Required. 0.5 gm or 0.5 mL.

c. Test Duration. Five weeks for sensitization reaction, six weeks for final submission of tabulated data.

d. Preliminary Irritation Testing.

(1) Prior to the beginning of the sensitization procedure, two guinea pigs are treated to determine irritancy. The animals are shaved along midline of the back and receive neat, 10 percent, 1 percent, and 0.1 percent of the test material (0.05 mL vol). Injections are given intradermally using a 27 gauge needle.

(2) Animals are examined at 24 and 48 hrs, and the highest dose producing no irritation is the one selected for sensitization testing. Slight irritation is defined as a numerical score of 25 to 50 using Tables 1 and 2 for scoring. In cases of severe irritation, lower doses may have to be selected and two more guinea pigs used.

e. Sensitization Procedure.

(1) Fifteen guinea pigs are now required for each compound to be tested. The animals are tattooed with their number in the ear and are examined for general physical condition. Ten animals will be randomly selected and designated as the test group, with the remaining five serving as cage controls and not tested until the challenge injection. With each series of compounds to be tested, an additional 15 animals are needed as positive controls. These animals are treated with a 0.1 percent solution of dinitrochlorobenzene, a known sensitizer, using the same schedule as the other groups.

(2) The sensitization test is started on a Monday. All guinea pigs are weighed, clipped, and examined. An injection of 0.05 mL of the solution to be tested is injected intradermally into the upper right scapular area. An additional 0.05 mL of the diluent used is injected into the upper-left scapular area. Animals are scored at 24 and 48 hrs for irritation on both sides, using the numerical system provided in Table 1. These scores are then recorded on HSE-LT Form 55 (Appendix).

TABLE 1. GRADING OF SKIN REACTIONS IN THE GUINEA PIG SENSITIZATION TEST

The grading system is designed so that the intensity of the skin reaction is represented by a proportionate numerical value and also any reaction elicited by the vehicle ("control substance") is subtracted from the reaction produced by the test substance and the vehicle combined.

The product of the width and length (in mm) of the wheal is multiplied by the following reaction scores:

0 = needle puncture ("np") - no wheal
1 = very faint pink ("vfp") - no value is recorded for this reaction
2 = faint pink ("fp")
3 = pink ("p")
4 = red ("r")
5 = bright red ("R")
6 = edema - <1 mm in height ("e")
7 = edema - >1 mm in height ("E")
8* = necrosis - <1 sq. mm ("nec")
9* = necrosis - >1sq. mm ("NEC")

* The product of width and length of the necrotic area multiplied by 8 or 9 is added and is the numerical value of any of the foregoing reactions that are present.

TABLE 2. CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES*

The numerical values of the 24-hour readings are calculated from the following equations:

$G_2 - G_1 = a$

$G_4 - G_3 = b$

$b - a$ = final grade

Where G_1 = 24 hour reaction score from initial injection of vehicle

G_2 = 24 hour reaction score from challenge injection of vehicle

G_3 = 24 hour reaction score from initial injection of test substance

G_4 = 24 hour reaction score from challenge injection of test substance

The numerical values of the 48-hour readings are calculated from the following equations:

$G_6 - G_5 = c$

$G_8 - G_7 = d$

$d - c$ = final grade

Where G_5 = 48 hour reaction score from initial injection of vehicle

G_6 = 48 hour reaction score from challenge injection of vehicle

G_7 = 48 hour reaction score from initial injection of test substance

G_8 = 48 hour reaction score from challenge injection of test substance

A final grade of 25 or less indicates no sensitizing potential and a final grade of 100 indicates a moderate sensitization potential, to guinea pigs.

* The Landsteiner Guinea Pig Sensitization Test, as modified by the Chemical Hygiene Fellowship, Mellon Institute; July 1967.

(3) The sensitizing doses of 0.1 mL of the test solution are then injected into the clipped dorsal lumbosacral area on Wednesday, Friday, and Monday for the next 3 weeks until nine additional injections have been given. Care should be given to prevent injection of these solutions into the same area as prior doses. The guinea pigs are clipped over the scapular and lumbosacral area each week.

(4) Following the ninth sensitizing dose (0.1 mL) which will occur on a Monday, the animals are rested for 2 weeks. On the fourteenth day all guinea pigs are again clipped, weighed, and closely examined prior to the challenge injection. The challenge dose (0.05 mL) is then administered into the right scapular area as before, with the diluent injection given to the left. Irritation scores are read at 24 and 48 hrs and recorded on HSE-LT Form 55, using Tables 1 and 2.

(5) The groups that were labeled as cage controls now receive their first injection (0.05 mL), in the same manner as the test group. The positive cage controls will receive the known sensitizer, while the others will be given the corresponding test solutions. These groups of animals are scored in the same manner as the test groups, and are used to determine the effect of age and compound viability.

(6) Compounds are then reviewed using Table 2 to determine their relative sensitizing potential.

f. Materials and Methods.

(1) In most cases, guinea pigs used in this procedure are injected intradermally with the test material. All animals are injected using a 1 mL tuberculin syringe and a 27 gauge, 1/4 inch needle. Compound dilutions for this test will be made with normal saline when possible, and a hot plate and stirring bar may be utilized for mixing the solutions and warming them (not to exceed 50°C). Powders and liquids found to be insoluble in saline can frequently be initially dissolved or suspended in propylene glycol.

(2) In cases of solid materials, i.e., cloth, plastics, 1 cm^2 pieces are applied to the back with a drop of saline between the material and the skin to insure intimate contact.

(3) Propylene glycol can be ordered through the Federal supply system, NSN 6505-00-038-4150. Saline is available from Abbott Laboratories, stock No. 8817. Needles and syringes can be obtained from Becton-Dickinson Company, stock Nos. 5602 and 3201, respectively.

5. APPROVALS.

a. This study will run in accordance with 21 CFR 58, Good Laboratory Practices, and as approved by Animal Use Review Committee.

Mack A. Holt

MACK A. HOLT, DVM
CPT, VC
Chairman, Animal Use Review Committee

b. This SOP has been reviewed and approved by the USAEHA Quality Assurance Office. The Quality Assurance Office inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Paul V. Sneeringer

PAUL V. SNEERINGER, Ph.D.
Chief, Analytical Quality
Assurance Office

c. Designated Toxicology Division personnel will be responsible for the performance of this SOP.

ARTHUR H. MCCREESH, Ph.D.
Chief, Toxicology Division

d. This SOP was prepared by:

John G. Harvey

JOHN G. HARVEY
Biological Laboratory Technician
Toxicology Division

APPENDIX

GUINEA PIG SENSITIZATION TEST

Chemical Name: _____ Study No. _____

Procedure: _____ Technician: _____

_____ Physical State of Compound: _____

Date Test Started: _____ Solubility: _____

24-HOUR READINGS AND GRADES

Guinea Pig Number	Guinea Pig Wts., Gms. Init.	Guinea Pig Wts., Gms. Final	Diluent Substance: Init. Inject. Date	G_1	Diluent Substance: Challenge Injection Date	G_2	Diluent Substance: $a = (G_2 - G_1)$	Test Substance: Init. Inject. Date	G_3	Test Substance: Challenge Injection Date	G_4	Test Substance: $b = (G_4 - G_3)$	Final Grade (b-a)

Comments (Conclusions on back)

HSE-LT Form 50, 1 Jun 80

Replaces USAEHA Form 55, 12 Aug 74, which will be used.

48-HOUR READINGS AND GRADES

Guinea Pig Number	Diluent Substance					Test Substance					Final Grade (d-c)
	Init. Inject. Date	G_5	Challenge Injection Date	G_6	c = (G_6-G_5)	Init. Inject. Date	G_7	Challenge Injection Date	G_8	d = (G_8-G_7)	

Conclusions: